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SPECTRAL SENSITIVITY OF THE SPINY LOBSTER, *PANULIRUS ARGUS*

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Abstract

The spectral sensitivity of receptor cells in the eye of the spiny lobster *Panulirus argus* was measured with intracellular electrodes. All cells sampled were maximally sensitive at about 510 nm. Several of these retinular cells were filled with Lucifer Yellow-CH from the recording electrode, and subsequent histological examination showed that they contributed to the main rhabdom and their axons terminated in the *lamina ganglionaris*. Microspectrophotometry of the main rhabdom revealed a single visual pigment with λ_{max} at about 510 nm. Using extracellular recording and selective adaptation with colored lights, however, a second receptor type was unmasked, with peak sensitivity at 370 nm in the UV. Indirect evidence suggests that it is the small, eighth retinular cell located distal to the main rhabdom, which has been shown in crayfish to contain a visual pigment maximally sensitive at short wavelengths and to make a comparably small contribution to the ERG.

Introduction

The structural organization of the compound eye of the lobster, *Panulirus*, has been studied by several researchers (Parker, 1891; Eguchi and Waterman, 1966, Meyer-Rochow 1975). The adult eye is of superposition (scotopic or clear zone) morphology, in which the crystalline cones are separated from the retinular cell layers by a wide transparent region. Seven retinular cells contribute alternating layers of microvilli to a central rhabdom, which has an unusual shape in which the lobed proximal region is more than 10 times the diameter of the thin distal neck. The eye also contains an eighth retinular cell which lies distal to the main rhabdom.

Physiological studies of the eye of *Panulirus argus* have been few, although the animal exhibits a repertoire of visually oriented behaviors. Waterman and Wiersma (1963) recorded the ERG, and Fernandez (1965) extracted a visual pigment with a λ_{max} at ~504 nm. The nature of the rhabdom of *Panulirus longipes* and its possible insensitivity to the plane of polarization was discussed by Meyer-Rochow (1975).

Panulirus argus is largely nocturnal, foraging in shallow water around tropical coral reefs (Kanciruk and Herrnkind, 1978). A notable exception to this general pattern occurs every fall when the entire population exhibits diurnal activity and engages in a highly organized migration of several km. Declining water temperatures in autumn trigger the migration, which lasts for several days. The animals form single file columns of up to 60 individuals and maintain the queues for the duration of their migration. The biological reason for this mass migration is unknown, as is the method of orientation while they travel.

Materials and Methods

Animals

Specimens of the spiny lobster *Panulirus argus* were obtained from Carolina Biological Supply. They were kept for as long as two months under overhead fluorescent

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lights on a 12 h light:12 h dark cycle in aerated sea water aquaria and fed fresh mussels. The temperature of the room was maintained at 30°C.

Electrical recording

For electrophysiological recording, the animal was dark-adapted overnight, and the eyestalk was excised and all structures distal to the retinal layer were dissected free under dim red light. After being mounted in the recording chamber and submerged in saline, the preparation was dark-adapted for at least one hour. The eye was then pinned to a cork base in a lucite chamber filled with MBL crustacean saline (Pantin, 1934). Intracellular recording electrodes had 45–60 MΩ resistances when filled with 3 *M* KCl and immersed in marine crustacean saline. The reference electrode was a chlorided silver wire in a glass capillary tube which was filled with 2% agar in 3 *M* KCl. Micropipette electrodes were also used for measurement of the ERG by recording subcorneally in extracellular space. Electrical responses were viewed and photographed on the face of an oscilloscope.

Optical stimulator

Light (10 nm half band width) from a grating monochromator (Bausch and Lomb) supplied by a 500 W tungsten-iodide lamp was focused onto the eye using a combination of glass lenses. Intensity was controlled with a pair of counter-rotating quartz-inconel optical wedges. An electromagnetic shutter delivered flashes of light of 100 ms duration. The system was calibrated with a photodiode of known sensitivity (United Detector Technology).

In experiments involving selective adaptation, the adapting source was a microscope lamp fitted with appropriate filters. The light source was focused onto the eye from the same direction as the stimulus by using a beam splitter and lenses.

Spectral sensitivity

Spectral sensitivity was assessed by stimulating the eye with a series of monochromatic lights (10 nm half-band width) at 5–40 nm intervals between 350 nm and 600 nm. The 5 nm intervals were used around the sensitivity maximum, but not in all experiments. Stimuli were 100 ms, separated by dark periods of up to 1 minute. The spectrum was swept in both directions in order to compensate for any systematic drift in the sensitivity of the preparation. At each wavelength, several stimuli were presented and the wedges adjusted until the criterion response was elicited (using either intracellular responses or the ERG).

Selective adaptation

For selective adaptation, an appropriate filter was placed in the adapting light source: Corning CS3-71 (λ's > 475 nm) for the yellow light adaptation and Corning CS5-60 (λ_{max} = 415 nm, 90 nm band width at 50% maximal transmission) for blue light adaptation. Relative to the maximum value at about 440 nm, the quantum flux of the blue adapting light was about 0.24 at 380 nm and 0.06 at 500 nm. The wedge was opened to allow the full light intensity at 350 nm to be used as a stimulus. The adapting light was then turned on and the intensity increased until the responses at 350 nm were reduced to the criterion amplitude. The spectral sensitivity was then assessed by the method described in the previous paragraph.

Lucifer Yellow-CH fills

For marking individual cells, iontophoretic recording electrodes were filled at the tip with a 5% aqueous solution of Lucifer Yellow-CH (Sigma) and backfilled with 0.1 *M* LiCl. Cells were iontophoretically injected with dye by passing 100 ms, 10 nA hyperpolarizing pulses at a rate of 5 s^{-1} for 5 to 6 minutes.

Retinas were dissected from their eyestalks and fixed overnight in a solution of 4% formaldehyde (from paraformaldehyde) in a 0.1 *M* sodium phosphate buffer (pH 7.4) at 5°C, dehydrated through an alcohol series, embedded in paraplast (m.p. 56–58°C) and sectioned at 5 μm. The serial sections were examined under a Zeiss fluorescence microscope using a mercury lamp and a 430 nm interference filter in the excitation path. The image was passed through 460 and 530 nm long pass barrier filters and photographed.

Microspectrophotometry

Rhabdoms from dark-adapted lobsters were isolated by dissecting the retinal layer under dim red light. The tissue was crushed with a stout glass rod in 1 ml of MBL crustacean saline (Pantin, 1934) at 0°C. No fixative was present. Drops of the suspension were placed in a ring of silicone grease and sealed between coverslips for study by microspectrophotometry as described below, and measurements were made at room temperature (22° ± 2°C). Solutions were buffered with 0.04 *M* HEPES (N-2-hydroxyethylpiperaxine-N′-2-ethane sulfonic acid), but in some experiments, small droplets of 0.01 *N* NaOH were added to move the pH to more extreme basic values.

A laterally incident microbeam that was rectangular and measured 7 × 2 μm in the plane of the specimen was used for spectral recording. This microbeam was produced by a dual beam microspectrophotometer with a reference beam external to the microscope. The light source was a 6 V, 100 W tungsten lamp, used in conjunction with a Bausch and Lomb 250 mm focal length monochromator with the grating ruled at 600 lines per mm. For the measuring beam, an image of an aperture was projected onto the specimen with a Zeiss X32 Ultrafluar glycerin immersion objective mounted on the condenser carriage of the microscope. An identical lens was employed in the collecting system. The detector was an EMI 9558 QA photomultiplier tube. Further details can be found in Goldsmith (1978).

The scans were under computer program control and spectra were digitized at 1 nm intervals. The duration of a single scan was ~30 seconds. No photoconversion caused by the measuring beam was observed when the organelle was scanned a second time. Each interval reading was an average of 25 interrogations of the A/D converter.

Results

Intracellular potentials were recorded from approximately five hundred retinular cells in the isolated eye cup preparation of the lobster. Figure 1 shows average spectral sensitivity curves derived from intracellular recordings of cells from dark-adapted animals, based on depolarizing criteria of 5 mV (n = 25) and 2 mV (n = 6). Both curves have their maxima 510 ± 5 nm. Yellow and blue light adaptation of the eye failed to alter the shape of the spectral sensitivity curve derived from intracellular responses, providing no evidence for electrical coupling between receptors of different spectral type (Fig. 2).

Lucifer fills of the 510 nm receptor revealed that it is associated with the main rhabdom and synapses in the *lamina ganglionaris*.

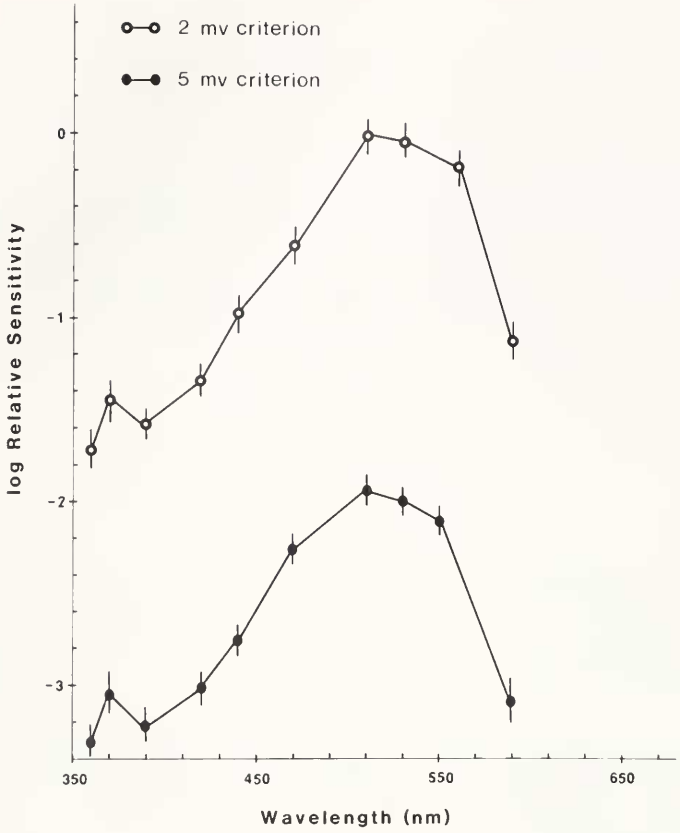

FIGURE 1. Spectral sensitivity curves of photoreceptor cells of *Panulirus argus* based on intracellular recordings. The upper curve is an average of 6 cells and is log reciprocal photon flux (in relative units) for a criterion response of 2 mV; the lower curve is an average of 25 cells and is based on a criterion depolarization of 5 mV. In both cases the peak sensitivity is at about 510 nm. Error bars indicate ±s.e.m.

As only a single spectral class of receptor was found in intracellular recording, experiments using the ERG were employed in order to unmask any possible contributions of other receptor types. The spectral sensitivity of the ERG of the dark-adapted eye was similar to the spectral sensitivity functions of individual cells, and the response-energy functions were parallel to each other at different wavelengths (not shown). But when the eye was adapted with colored lights and a very small criterion response employed, evidence appeared for a second type of receptor. The upper curve in Figure 3 is a spectral sensitivity function based on a 25 μV criterion response of the ERG from a dark adapted eye. When the eye was adapted with blue or orange background lights, however, the sensitivity not only decreased, but the shape of the spectral sensitivity function changed as well. Adaptation to long wavelengths accentuated the secondary maximum at 370 nm, whereas adaptation to short wavelengths abolished it. This effect was observed only with very small criterion responses ($\sim$25 μV). These experiments show the presence of two types of receptors, one having a maximum sensitivity in the green (λ_{max} 510 nm) and the other type

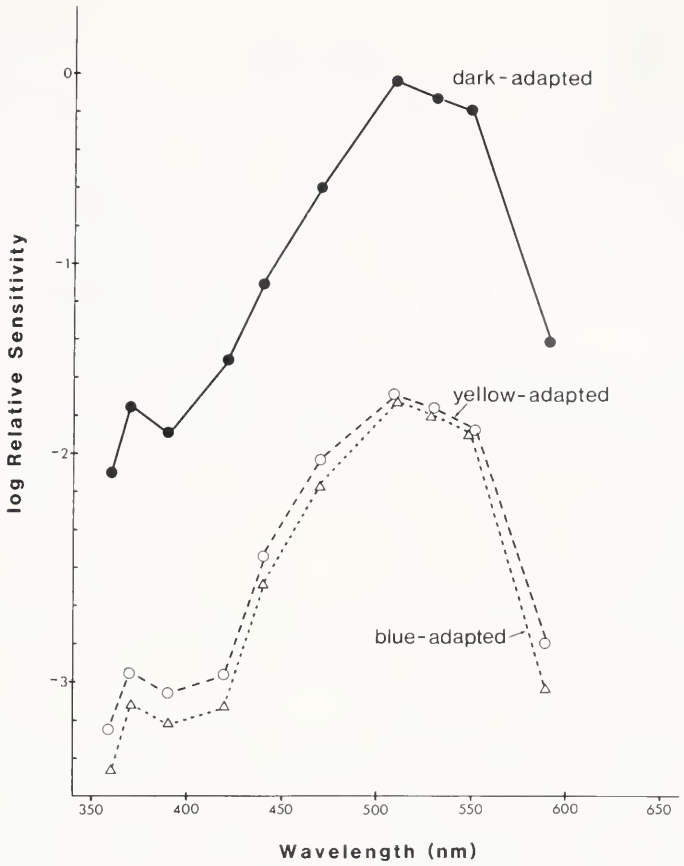

FIGURE 2. Spectral sensitivity of a dark adapted eye, derived from intracellular recording (filled circles, upper curve). The maximum sensitivity is at about 510 nm. When spectral sensitivity is measured in the presence of colored backgrounds, the shape of the spectral sensitivity function is unaltered (lower 2 curves).

having a maximum sensitivity in the ultraviolet (λ_{max} near 370 nm). The former seems the more abundant, as it dominates the sensitivity of the dark-adapted eye.

Microspectrophotometry of the main rhabdom demonstrated the presence of one pigment with a maximal absorption at 510 nm. Over 30 scans of randomly selected areas of main rhabdoms revealed only the 510 nm pigment. An example is shown in Figure 4. In order to obtain a difference spectrum, the pigment was bleached by orange light at pH 9, a treatment known to cause the photobleaching of crustacean rhodopsin (Bruno *et al.,* 1977). The metarhodopsin was not studied.

DISCUSSION

Panulirus rhodopsin in digitonin solution has λ_{max} at 504 nm (Fernandez, 1965), which is in reasonable agreement with both our electrophysiological and microspectrophotometric measurements. The data do not allow us to conclude that the 6 nm difference is real. Fernandez found the λ_{max} of *Panulirus* metarhodopsin at 495 nm.

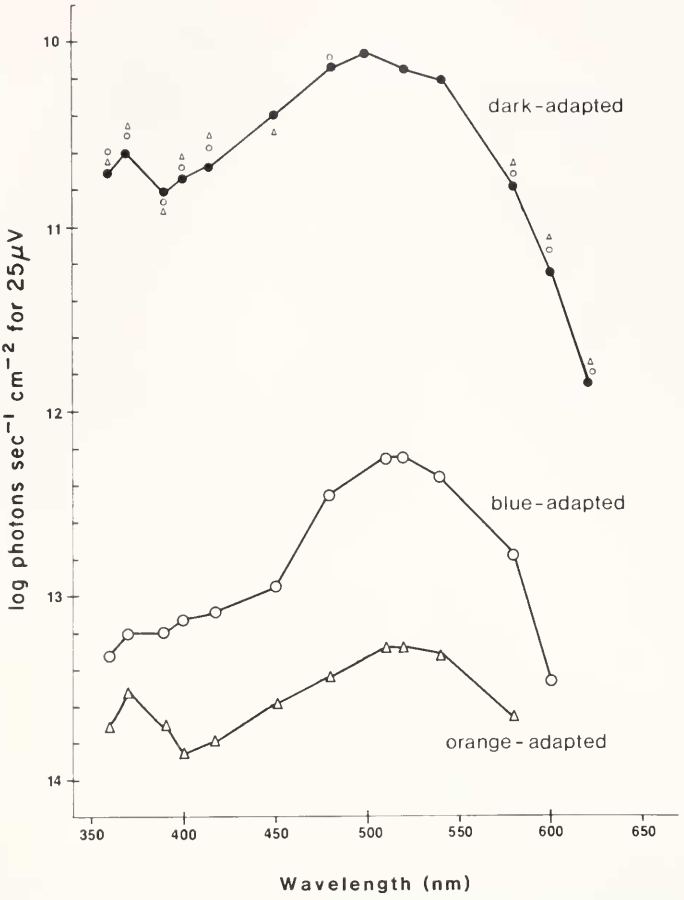

FIGURE 3. Spectral sensitivity of the dark-adapted eye, derived from extracellular recordings (filled circles and upper curve). The maximum sensitivity is at about 500 nm. When spectral sensitivity is measured in the presence of colored backgrounds, however, the shape of the spectral sensitivity function changes. This effect is observed only with small criterion responses, in this case 25 μV. The middle curve and large open circles show the suppression of the secondary maximum at 370 nm brought about by adaptation to blue light. Conversely, the lower curve and large open triangles show the enhancement of the 370 nm peak produced with an orange background. Small open circles and triangles show the recovery of sensitivity following the two adaptations.

The crayfish *Orconectes* and *Procambarus* (Goldsmith and Fernandez, 1968; Wald, 1968; Nosaki, 1969; and Waterman and Fernandez, 1970) and the prawn *Palaemonetes* (Goldsmith and Fernandez, 1968; Wald and Seldin, 1968) have two spectral types of receptor in the eye. In the crayfish the sensitivity maxima are at 560 and 440 nm; in the prawn, at about 550 and 380 nm. In each species the short wavelength receptors contribute little to the ERG, and in the case of the crayfish, intracellular recording and dye marking, combined with microspectrophotometric measurements, have shown the 440 nm receptor to be the small, distal eighth cell (Cummins and Goldsmith, 1981).

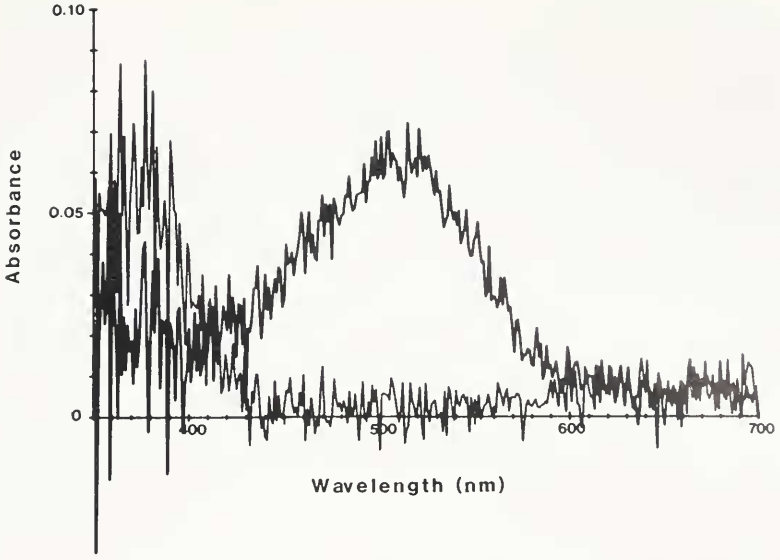

FIGURE 4. Microspectrophotometry of the main rhabdom reveals a visual pigment with peak absorption at about 510 nm (pH 7.5). At pH 9, several minutes exposure to a bright orange light causes the pigment to bleach to a photoproduct with peak absorption at about 370 nm, presumably retinaldehyde (left peak).

Extracellular recordings and selective adaptation experiments of the eye of *Panulirus* clearly indicate the presence of two spectral types of receptor in this species as well. The major contribution to the ERG comes from a receptor with a λ_{max} at 510 nm; a minor contribution is made by a receptor with a λ_{max} in the near UV. Furthermore, over 30 scans of random areas in the main rhabdom of the spiny lobster eye have shown the presence of only the 510 nm pigment. Although the 370 nm receptor was not picked up in the several hundred impalements made in all regions of the eye, we believe that, as in the crayfish, the short wavelength receptor is likely to be the eighth cell that is located distal to the main rhabdom. The fact that we failed to uncover the ultraviolet cell by intracellular recording could be due to several contributing factors, such as its small size, (5% of the rhabdom microvilli: Meyer-Rochow, 1975), its protection by its contiguous lenslet, or damage caused by removal of the more peripheral structures during preparation of the eye for recording.

ACKNOWLEDGMENTS

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